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RESEARCH ARTICLE

Enhanced biomass and photosynthetic pigment productivity of *Spirulina platensis* using a sustainable medium from chicken feather and *Moringa oleifera* leaf extract

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Abstract

Spirulina platensis biomass is a renewable resource for the production of high-quality commodities. However, substantial costs and nutrient availability limit large-scale production. Nutrients from agro-industrial by-products can be used as potential sources of nutrition to increase *S. platensis* production. This study evaluated the potential of chicken feathers (CF) and *Moringa oleifera* leaf extract (MLE) as an eco-friendly alternative medium for determining *S. platensis* biomass and photosynthetic pigment production. The culture medium was formulated by processing chicken feathers into CF media. Different MLE concentrations (0, 10, 15, and 20 g L^{-1}) were subsequently prepared in distilled water. *S. platensis* was cultured in cylindrical vessels under greenhouse conditions for 27 days. The CF media enriched with 15 g L^{-1} *M. oleifera* leaf extract (CF-MLE-15) presented the highest optical density (1.68 ± 0.03) and biomass dry weight (1.98 ± 0.05 g L^{-1}), outperforming the values observed in the standard (JM) and the control (CF-MLE-0) media. Moreover, the content and productivity of carotenoids and chlorophyll a were significantly higher ($p < 0.05$) in the CF-MLE-15 treatment than in the other CF-MLE and control treatments. Nonetheless, the contents of chlorophyll a (1.72 ± 0.12 mg L^{-1}), carotenoids (0.90 ± 0.003 mg L^{-1}), and phycobiliproteins in the standard medium (JM) exceeded those found in the CF-MLE treatments. Consequently, a CF medium supplemented with 15 g L^{-1} of *M. oleifera* leaf extract may be a viable and economical alternative for producing *S. platensis* biomass and photosynthetic molecules.

Keywords:
Spirulina platensis, biomass production, photosynthetic molecules, chicken feathers, *Moringa oleifera*

Highlights

- Chicken feathers and *Moringa oleifera* leaf extract as eco-friendly and alternative medium for *Spirulina platensis* biomass and photosynthetic pigment production
- Chicken feathers and *Moringa oleifera* leaf extract enhance *Spirulina platensis* growth, protein and photosynthetic pigment production
- Adequate ratio of chicken feathers to *Moringa oleifera* leaf extract leads to optimal *Spirulina platensis* biomass, protein and photosynthetic pigment production
- Chicken feathers and *Moringa oleifera* leaf extract provide a sustainable, cost-effective culture medium for *Spirulina platensis*, addressing agro-industrial waste challenges

Introduction

Microalgae have attracted global interest due to their potential uses in diverse sectors, including renewable energy, pharmaceuticals, nutraceuticals, food, and cosmetics [1]. Microalgae biomass is renewable, sustainable and cost-effective sources of bioactive photosynthetic pigments [2]. *Spirulina platensis* (*S. platensis*) a blue-green, filamentous, photosynthetic cyanobacterium within microalgae, is recognized as a highly promising species that thrives in aquatic habitats such as lakes, rivers and oceans [3]. *S. platensis* is particularly noteworthy for its high nutritional value and therapeutic properties. *S. platensis* content a high percentage of protein (50-70%), polyunsaturated fatty acids (α-linolenic acid, γ-linolenic acid), photosynthetic molecules (chlorophylls, carotenoids and phycobiliproteins), vitamins (provitamins A, vitamin B1) and minerals (iron, calcium, magnesium, zinc and potassium) [4, 5]. Indeed, the Food and Agriculture Organization has recognized it as a vital and integrated food resource for humanity in the 21st century [6]. *S. platensis* has therapeutic properties, including anti-aging, anti-cancer, immunomodulatory, antibacterial, and anti-inflammatory effects [7, 8].

S. platensis is highly adaptable and can thrive under various nutritional conditions. [9]. Large-scale cultivation of *S. platensis* commonly involves the application of Jourdan and Zarrouk media as nutritional resources. These two inorganic media are complex, expensive, and not widely available [10]. To reduce the production costs of *S. platensis*, researchers are exploring more cost-effective and readily available alternative culture media [11-13]. Studies have investigated the use of agro-industrial by-products, such as chicken feathers and *M. oleifera* leaves, as potential sources of nutrition for microalgae culture [14, 15]. *M. oleifera* leaves contain vitamins and essential micronutrients such as magnesium, potassium, iron and copper, which are necessary for microalgal growth [16, 17]. Chicken feathers provide cost-effective nutrient-rich sources of nitrogen, phosphorus, calcium, and magnesium for microalgae cultivation [18, 19]. The complementary nutrient profiles from these two agro-industrial by-products synergistically enhance microbial metabolic activity, and stimulate biomass production [20, 21]. Despite their inherent nutrient richness, no prior study has investigated the potential of chicken feathers and *M. oleifera* leaf extracts as an alternative medium for *S. platensis* biomass and photosynthetic pigment production.

The novelty of this study lies in the use of agro-industrial by-products, such as chicken feathers and *Moringa oleifera* leaves, as prospective substrates for cultivating microalgae, including *Spirulina platensis*. The strategy helps mitigate production costs and alleviates agro-industrial waste management challenges. The use of chicken feathers and *Moringa oleifera* leaf extract in microalgae cultivation also provides an eco-friendly alternative to synthetic media.

Thus, the current study investigated the potential of chicken feathers and *M. oleifera* leaf extracts as an eco-friendly alternative medium for producing *S. platensis* biomass and photosynthetic pigments. These findings highlight the promising use of these alternative media for the production of *S. platensis* biomass and photosynthetic pigments.

Materials and Methods

Preparation of chicken feathers medium supplemented with *Moringa oleifera* leaf extract

The chicken feathers were collected as waste at a slaughterhouse in Douala, Cameroon. The samples were washed at 50 °C in 0.5% v/v H_2O_2 to remove residues of blood, faeces, fat, offal and sand. The washed chicken feathers were then rinsed and dried at 105 °C for 24 hours. Once dried, the branches were manually separated from the shaft and carbonized at 440 °C for 10 min in a muffle furnace to obtain ash [22]. Subsequently, 10 g of ash was pulverized in 100 mL of distilled water (1:10 w/v) and left to stand for 24 hours to create a homogenate of chicken feathers. After the homogenization process, the mixture was passed through the Whatman No. 2 filter paper. The filtrate collected was used as chicken feather medium (CF).

Fresh *M. oleifera* leaves were harvested at the SAGRIC farm in Douala, Cameroon. The leaves were cleaned and different quantities (20 g, 30 g, and 40 g) were mixed with 1 L of distilled water for 7 days under agitation using a homogenizer [15]. The suspension was subsequently filtered through sterile muslin cloth and Whatman No. 1 filter paper to eliminate solid particles. The filtrates obtained were diluted with an additional 1 L of distilled water to achieve final concentrations of 10, 15 and 20 g L^{-1} *Moringa oleifera* leaf extracts and sterilized at 121°C for 30 minutes. The obtained extracts were used as the *M. oleifera* leaf extract (MLE).

Microalgae and inoculum culture conditions

The strain of *S. platensis* used in the experiments was harvested from the algal culture collection of the SAGRIC farm in Douala, Cameroon. To produce the inoculum, the *S. platensis* strain was cultured in Jourdan's medium, which contained per liter: $NaHCO_3$ (8 g), NaCl (5 g), KNO_3 (2 g), $MgSO_4$ (0.16 g), $(NH_4)_2HPO_4$ (0.12 g), $(NH_2)_2CO$ (0.05 g), $FeSO_4$ (0.02 g), and $CaCl_2$ (0.02 g). The culture of the inoculum was maintained at 28 ± 0.5 °C, under alkaline conditions (pH = 9.0 ± 0.4) in a greenhouse with white LED tube lamps (200 µmol photons m^{-2} s^{-1}) and constant aeration supplied by a membrane pump (Laboport KNF, Germany).

Experimental design

The study used a systematic methodology, wherein *S. platensis* was cultivated in chicken feathers medium (CF). Culture media were prepared by supplementing CF medium with 0, 10, 15 or 20 g L^{-1} of MLE for CF-MLE-0 (control), CF-MLE-10, CF-MLE-15 or CF-MLE-20 respectively. Jourdan's medium served as the established standard medium (Table 1). The microalgae *S. platensis* was grown in 20 L cylindrical vessels set in a greenhouse maintained at 28 ± 0.5 °C, under white LED illumination (200 µmol photons m^{-2} s^{-1}) and continuous aeration supplied by a membrane pump. A 10% inoculum (Inoculation volume/Medium volume) was used at the beginning of the cultivation experiment. The salinity and the pH were adjusted to 11.4 ± 0.5 PSU with NaCl and 9.0 ± 0.4 with 2 N NaOH respectively. The trials were repeated thrice during the 27 days of experimentation.

Table 1. The chemical compound of the chicken feathers medium supplemented with *M. oleifera* leaf extract and Jourdan medium employed for the production of *Spirulina platensis* biomass

Jourdan standard medium (JM)		Chicken feathers medium supplemented with *M. oleifera* leaf extract		
Constituents	Concentration (g L^{-1})	Treatments	Constituents	Concentration (g L^{-1})
$(NH_2)_2CO$	0.05		$NaHCO_3$ *	8
$(NH_4)_2HPO_4$	0.12		NaCl *	5
KNO_3	2		CF *	10
$MgSO_4$	0.16			
$CaCl_2$	0.02	CF-MLE - 0 (control)		0
$FeSO_4$	0.02	CF-MLE - 10		10
NaCl	5	CF-MLE - 15	MLE*	15
$NaHCO_3$	8	CF-MLE - 20		20

* Note: CF (chicken feathers medium), MLE (*M. oleifera* leaf extract). $NaHCO_3$ and NaCl were added in all the treatments of the *M. oleifera* leaf extract.

Estimation of the eco-friendly chicken feathers medium supplemented with *Moringa oleifera* leaf extract

The expense associated with chicken feather medium enriched with *Moringa oleifera* leaf extract was calculated by taking into account the concentration and cost of every substance employed in the production of 1 kg of the material. Taxes, energy consumption and transportation expenditures are not included in the analysis. The prices for all the chemicals utilized were sourced from previous research reported by Magwell *et al.* [23]. (Table 2).

Table 2. Estimation of the expenditure of the eco-friendly chicken feathers medium supplemented with *Moringa oleifera* leaf extract and Jourdan standard medium employed for the production of *Spirulina platensis* biomass

Jourdan standard medium (JM)		Chicken feathers medium supplemented with *Moringa oleifera* leaf extract	
Components	Price (US Kg^{-1})	Components	Price (US Kg^{-1})
$(NH_2)_2CO$	342.13	$NaHCO_3$	69.08
$(NH_4)_2HPO_4$	130.75	NaCl	49.90
KNO_3	119.42	CF *	0
$MgSO_4$	233.17	MLE *	0
$CaCl_2$	128.57		
$FeSO_4$	82.92		
NaCl	49.90		
$NaHCO_3$	69.08		
Total price	1155.94	Total price	118.98

* Note: The chicken feathers and *Moringa oleifera* leaf used to prepare CF (chicken feathers medium) and MLE (*Moringa oleifera* leaf extract), respectively were freely collected as waste from a slaughterhouse and the SAGRIC farm in Douala, Cameroon.

Biomass and growth rate assessment

S. platensis samples were collected every 3 days to evaluate the dry biomass and cell optical density. The optical density of the cells was measured at 680 nm via a UV-VIS spectrophotometer (UV-VIS Spectrophotometer BK-S380, China). The determination of dry biomass was conducted through the filtration of 20 mL samples using 47 mm GF/C glass fiber filters (X_1, g). The cells within the filters were rinsed twice with distilled water, oven-dried at 50 °C for 24 hours and subsequently weighed (X_2, g). The dry biomass was calculated per the equation (1):

$$\text{Dry biomass } (X, \text{ g } L^{-1}) = [(X_1 - X_2) \times 1000] / 20 \quad (1)$$

The biomass productivity (P_x) and growth rate (μ) of *S. platensis* were determined from equations (2) and (3), respectively:

$$\text{Biomass productivity } (P_x, \text{ g } L^{-1} d^{-1}) = [X_f - X_0] / t \quad (2)$$

$$\text{Growth rate } (\mu, d^{-1}) = [\ln X_f - \ln X_0] / t \quad (3)$$

X_0 and X_f represent the dry biomass at the initiation and termination of the cultivation process (t), respectively.

Upon completion of the exponential growth phase (21 days), *S. platensis* samples were collected to assess protein, cysteine and pigment contents.

Biochemical analysis

Photosynthetic pigments

Chlorophyll and carotenoid contents

Chlorophyll a, b, and carotenoids were isolated from *S. platensis* biomass (2 mg) through the addition of 1 mL of 90% acetone in the absence of light for 24 hours at 4 °C. After centrifugation for 15 min at 5000 rpm (Sigma 1-15K Germany), the supernatant was harvested from each sample. The absorbances of chlorophyll a, b, and carotenoids were measured at 662 nm, 645 nm, and 470 nm, respectively. The contents of chlorophyll and carotenoids were assessed using the extinction coefficient in acetone and calculated from equations (4), (5) and (6) [24].

$$\text{Chl a (mg L}^{-1}) = 11.24 \times OD_{662} - 2.04 \times OD_{645} \quad (4)$$

where Chl a is chlorophyll a, OD_{662} denotes the optical density at 662 nm, and OD_{645} indicates the optical density at 645 nm.

$$\text{Chl b (mg L}^{-1}) = 20.13 \times OD_{645} - 4.19 \times OD_{662} \quad (5)$$

where Chl b is chlorophyll b, OD_{645} denotes the optical density at 645 nm, and OD_{662} signifies the optical density at 662 nm

$$\text{Carotenoids (mg L}^{-1}) = [(1000 \times OD_{470}) - (1.90 \times \text{Chl a}) - (63.14 \times \text{Chl b})] / 214 \quad (6)$$

where OD_{470} represents the optical density measured at 470 nm, Chl a denotes chlorophyll a and Chl b is chlorophyll b.

Phycobiliproteins contents

The fresh *S. platensis* biomass was mixed with phosphate buffer (0.05 M, pH 6.7) at a ratio of 1:3 (w/v) through three successive freezing and thawing cycles for 24 h at 4 °C, in the dark. Afterwards, the mixtures were centrifuged for 20 min at 5000 rpm and the supernatants were assessed through a UV-VIS spectrophotometer. The absorbance values of C-phycocyanin, allophycocyanin and phycoerythrin were recorded at 562 nm, 615 nm, and 652 nm respectively. The contents of C-phycocyanin C, allophycocyanin, and phycoerythrin were carried out through the application of equations (7), (8) and (9) [25]:

$$\text{C-Phycocyanin (C-PC)} = [OD_{615} - 0.474 \times OD_{652}] / 5.34 \quad (7)$$

$$\text{Allophycocyanin (APC)} = [OD_{652} - 0.208 \times OD_{615}] / 5.09 \quad (8)$$

$$\text{Phycoerythrin (PE)} = [OD_{562} - (2.41 \times \text{C-PC}) - (0.849 \times \text{APC})] / 9.62 \quad (9)$$

Productivity of chlorophyll, carotenoids and phycobiliproteins

The productivities of chlorophyll a (P_{Chla}, $mg.L^{-1}.d^{-1}$), chlorophyll b (P_{Chlb}, $mg.L^{-1}.d^{-1}$), carotenoids (P_{Cat}, $mg.L^{-1}.d^{-1}$), C-phycocyanin ($P_{C\text{-}PC}$, $mg.L^{-1}.d^{-1}$), allophycocyanin (P_{APC}, $mg.L^{-1}.d^{-1}$) and phycoerythrin (P_{PE}, $mg.L^{-1}.d^{-1}$) were assessed based on dry biomass, as outlined in equations (10), (11), (12), (13), (14) and (15) respectively. Where X_f, Chl a, Chl b, Cat, C-PC, APC, PE, and Δt represent dry biomass, chlorophyll a, chlorophyll b, carotenoids, C-phycocyanin, allophycocyanin, phycoerythrin, and cultivation time, respectively.

$$P_{Chla}\ (g.L^{-1}.d^{-1}) = [Chl\ a \times X_f] / \Delta t \quad (10)$$

$$P_{Chlb}\ (g.L^{-1}.d^{-1}) = [Chl\ b \times X_f] / \Delta t \quad (11)$$

$$P_{Cat}\ (g.L^{-1}.d^{-1}) = [Cat \times X_f] / \Delta t \quad (12)$$

$$P_{C\text{-}PC}\ (g.L^{-1}.d^{-1}) = [C\text{-}PC \times X_f] / \Delta t \quad (13)$$

$$P_{APC}\ (g.L^{-1}.d^{-1}) = [APC \times X_f] / \Delta t \quad (14)$$

$$P_{PE}\ (g.L^{-1}.d^{-1}) = [PE \times X_f] / \Delta t \quad (15)$$

Protein and cysteine

The protein content was analysed using the method outlined by Bradford [26], which relies on the binding of proteins to a dye and the subsequent color change observed between 465 and 595 nm. For protein extraction, *S. platensis* dry biomass (5 mg) was mixed with 2 mL of 50 mM potassium phosphate buffer (pH 6.2) and left to interact for 10 min. The resulting mixture was collected after centrifugation at 3500 rpm for 10 min at 4 °C (Sigma 1-15K Germany). A volume of 0.1 mL of the supernatant was combined with 2 mL of the Bradford reagent (Sigma-Aldrich, Germany), and subsequently incubated in the dark for 10 min. Afterwards, the solution was transferred into cuvettes and the absorbance was recorded at 595 nm through a UV-VIS spectrophotometer (UV-VIS Spectrophotometer BK-S380, China).

The cysteine content was assessed through the method outlined by Gaitonde [27]. Cysteine extraction was performed by mixing *S. platensis* dry biomass (5 mg) with 5 mL of 80% ethanol and centrifuging it at 3000 rpm for 10 min. Cysteine crude extract (0.15 mL) was mixed with 0.35 mL of acidic ninhydrin reagent [1,3 % (w/v) ninhydrin in 1:4 HCl:CH_3COOH conc] (Sigma-Aldrich, Germany). The mixture was homogenized and heated at 100 °C for 10 min, then cooled on ice. After adding 1 mL of 95% ethanol, the optical density was assessed at 560 nm against a blank containing 80% ethanol instead of the cysteine crude extract.

Data Analysis

All the experiments were carried out in triplicate, and the results were presented as the means ± standard deviations. The statistical analysis was performed using IBM SPSS Statistics 26 and GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) through analysis of variance (ANOVA). Multiple comparisons of means for different essays

were executed through the Tukey test with a confidence level set at 95%. A statistically significant difference was identified when $p < 0.05$.

Results and Discussion

Effects of chicken feathers medium supplemented with *Moringa oleifera* leaf extract on *Spirulina platensis* growth performance

The variations in the cell density and dry biomass of *S. platensis* cultivated in CF medium enriched with *M. oleifera* leaf extract are illustrated in Figures 1A and 1B. An exponential increase in cell density and dry biomass was noted from day 1 to day 21. However, a significant decrease was observed from day 21 to day 27, especially in the control medium with chicken feathers extract, which resulted in reduced growth from day 9 onwards. The chicken feathers medium supplemented with 15 g L^{-1} *M. oleifera* leaf extract (CF-MLE-15) presented significantly ($p < 0.05$) higher cell density (1.68 ± 0.03) and dry biomass (1.98 ± 0.05 g L^{-1}) than standard Jourdan medium (JM), with a cell density and dry biomass of 1.58 ± 0.05 g L^{-1} and 1.83 ± 0.04 g L^{-1}, respectively. In contrast to the other treatments, the chicken feathers medium without the presence of *M. oleifera* leaf extract (CF-MLE-0) showed the lowest cell density (0.50 ± 0.03) and dry biomass (0.42 ± 0.06 g L^{-1}) of *S. platensis* (Table 3). These results lie in the nutrients (nitrogen, potassium, phosphorus, calcium, sodium and magnesium) found in the leaves of *M. oleifera* and chicken feathers, which enhanced cell growth and carbon metabolism during the photosynthetic activity of *S. platensis* [22, 28, 29]. Therefore, CF-MLE-15 will be more conducive to better growth and consequently higher cell density. The findings presented a higher level of efficacy than those reported by Wamba *et al.* [15] for a medium containing *M. oleifera* leaf extract enriched with either kanwa or sodium bicarbonate. The variation observed may be related to the formulation of the medium and the duration of the cultivation.

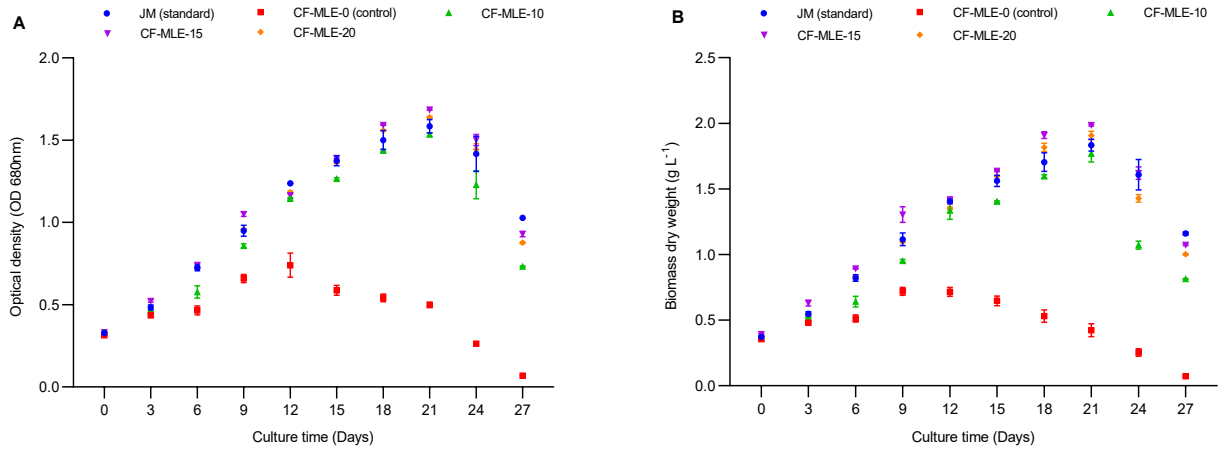

Figure 1. Growth performance of *S. platensis* in chicken feathers medium enriched with *M. oleifera* leaf extract (CF-MLE) and Jourdan medium (standard). (A) Optical density, (B) Biomass dry weight. The data are presented as means ± standard deviations (n = 3). The bars represent the standard deviation.

Biomass productivity (Px) and growth rate (μ) showed an upward trend in chicken feathers media with increasing concentration of *M. oleifera* leaf extract. It was observed that the standard Jourdan medium resulted in the highest biomass productivity (0.032 ± 0.003 g L^{-1} d^{-1}) and growth rate (0.040 ± 0.001 d^{-1}). Nonetheless, the CF-MLE-15 medium significantly ($p < 0.05$) increased the biomass productivity (0.028 ± 0.002 g L^{-1} d^{-1}) and growth rate (0.038 ± 0.001 d^{-1}) compared with those of the different chicken feathers media supplemented with *M. oleifera* leaf extract and the control medium (- 0.011 ± 0.001 g L^{-1} d^{-1} and - 0.057 ± 0.002 d^{-1}) (Table 3). These findings may be explained by the supply of necessary nutrients from *M. oleifera* leaf and chicken feather extracts during *S. platensis* cultivation. These results are congruent with the findings of Suparmaniam *et al.* [30], emphasizing the essential role of chicken feather nutrients in promoting microalgal growth.

Table 3. Optical density ($OD_{680\ nm}$), dry biomass (X), biomass productivity (P_x) and growth rate (μ_m) of *S. platensis* after 27 days of cultivation in chicken feathers medium enriched with *M. oleifera* leaf extract (CF-MLE)

Culture medium	Treatments (g L^{-1})	OD (680 nm)	X (g L^{-1})	P_x (g L^{-1} d^{-1})	μ (d^{-1})
Jourdan (standard)	JM	1.58 ± 0.05^b	1.83 ± 0.04^b	0.032 ± 0.003^a	0.040 ± 0.001^a
Chicken feathers supplemented with *M. oleifera* leaf extract	CF-MLE - 0 (control)	0.50 ± 0.03^c	0.42 ± 0.06^c	- 0.011 ± 0.001^d	- 0.057 ± 0.002^c
	CF-MLE - 10	1.53 ± 0.04^b	1.77 ± 0.06^b	0.018 ± 0.001^c	0.029 ± 0.001^b
	CF-MLE - 15	1.68 ± 0.03^a	1.98 ± 0.05^a	0.028 ± 0.002^b	0.038 ± 0.001^a
	CF-MLE - 20	1.63 ± 0.04^a	1.90 ± 0.04^a	0.025 ± 0.002^b	0.036 ± 0.001^a

The data are presented as the means ± standard deviations. The means with matching superscript letters ($a > b > c > d$) within the same column are not significantly different ($p > 0.05$) according to the Tukey test.

Effects of chicken feathers medium supplemented with *Moringa oleifera* leaf extract on the chlorophyll and carotenoid contents and productivity of *Spirulina platensis*

The contents of carotenoids, chlorophyll a, and chlorophyll b in *S. platensis* biomass cultivated from chicken feathers supplemented with various concentrations of *M. oleifera* leaf extract are presented in Table 4 and Figure 2. The variations in the carotenoid and chlorophyll a and b contents were correlated with the *M. oleifera* leaf extract concentrations in the medium. Supplementation of the chicken feathers medium with 15 g L^{-1} *M. oleifera* leaf extract (CF-MLE-15) led to a significant ($p < 0.05$) increase in the carotenoid (0.82 ± 0.02 mg L^{-1}), chlorophyll a (1.67 ± 0.07 mg L^{-1}) and chlorophyll b (1.20 ± 0.09 mg L^{-1}) contents. Nevertheless, the Jourdan standard medium presented significantly higher ($p < 0.05$) contents of carotenoids (0.90 ± 0.003 mg L^{-1}), chlorophyll a (1.72 ± 0.07 mg L^{-1}) and chlorophyll b (1.33 ± 012 mg L^{-1}) than the other treatments. Similarly, the productivity of chlorophyll and carotenoids of *S. platensis* showed a downward trend with the *M. oleifera* leaf extract concentration in the chicken feathers medium (Table 5). The productivity of

chlorophyll and carotenoids of *S. platensis* was significantly higher ($p < 0.05$) in CF-MLE-15 with 0.16 ± 0.001 g L^{-1} d^{-1} and 0.11 ± 0.002 g L^{-1} d^{-1}, respectively than the control with 0.02 ± 0.002 g L^{-1} d^{-1} and 0.01 ± 0.001 g L^{-1} d^{-1}, respectively (Table 5). The range of chlorophyll a, b and carotenoids in the current study is consistent with that reported by Herrera *et al.* [31], who reported values between 0.83 and 2.66 mg L^{-1}, using Schlösser and whey media under mixotrophic conditions as a substitute for synthetic media. This increase is attributed to the potassium, calcium, and iron provided by chicken feather and *M. oleifera* leaf extracts, which activated metabolic enzymes, regulated cellular processes, and maintained the structural integrity of the photosynthetic machinery [32, 33]. The ability to enrich chicken feathers medium with concentrations of 15 g L^{-1} MLE was noteworthy, as it influenced growth, dry biomass, and photosynthetic pigments. Conversely, at elevated MLE concentrations, cellular growth and photosynthetic performance were markedly diminished, leading to decreased levels of chlorophyll and carotenoids. Furthermore, Saad *et al.* [34] reported that the pigment content of *S. platensis* was lower under mixotrophic conditions than autotrophic conditions.

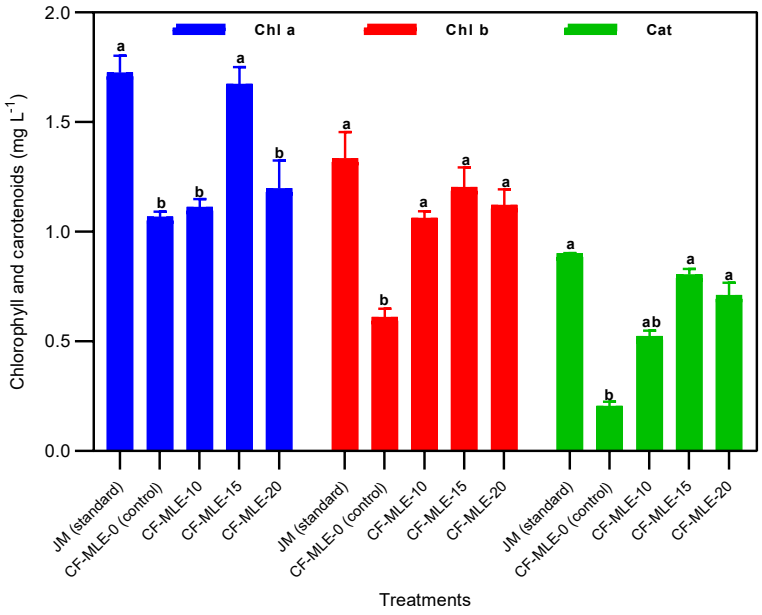

Figure 2. Chlorophyll a, b and carotenoid content in Chicken feathers medium supplemented with *M. oleifera* leaf extract (CF-MLE). The data are presented as means ± standard deviations. The bars represent the standard deviation. The means with matching superscript letters ($a > b > c > d$) within the same column are not significantly different ($p > 0.05$) according to the Tukey test.

Table 4. Chlorophyll (Chl a and b), total carotenoid (Cat), C-Phycocyanin (C-PC), Allophycocyanin (APC) and Phycoerythrin (PE) contents of *S. platensis* after 27 days of cultivation in chicken feathers medium enriched with *M. oleifera* leaf extract (CF-MLE)

Culture medium	Treatments (g L^{-1})	Chl a (mg L^{-1})	Chl b (mg L^{-1})	Cat (mg L^{-1})	C-PC (mg L^{-1})	APC (mg L^{-1})	PE (mg L^{-1})
Jourdan (standard)	JM	1.72 ± 0.12^{a}	1.33 ± 0.04^{a}	0.90 ± 0.003^{a}	0.86 ± 0.01^{a}	0.36 ± 0.02^{a}	0.20 ± 0.007^{a}
Chicken feathers supplemented with *M. oleifera* leaf extract	CF-MLE - 0 (control)	1.06 ± 0.02^{c}	0.61 ± 0.06^{d}	0.20 ± 0.001^{d}	0.30 ± 0.01^{c}	0.17 ± 0.01^{c}	0.11 ± 0.003^{b}
	CF-MLE - 10	1.11 ± 0.03^{b}	1.06 ± 0.06^{c}	0.52 ± 0.001^{c}	0.75 ± 0.01^{b}	0.26 ± 0.01^{b}	0.18 ± 0.005^{a}
	CF-MLE - 15	1.67 ± 0.05^{a}	1.20 ± 0.05^{b}	0.82 ± 0.002^{a}	0.77 ± 0.03^{b}	0.28 ± 0.03^{b}	0.19 ± 0.001^{a}
	CF-MLE - 20	1.19 ± 0.12^{b}	1.12 ± 0.04^{c}	0.71 ± 0.002^{b}	0.75 ± 0.01^{b}	0.25 ± 0.01^{b}	0.18 ± 0.005^{a}

The data are presented as means ± standard deviations. The means with matching superscript letters ($a > b > c > d$) within the same column are not significantly different ($p > 0.05$) according to the Tukey test.

Effects of chicken feathers medium supplemented with *Moringa oleifera* leaf extract on the phycobiliprotein content and productivity of *Spirulina platensis*

The phycobiliprotein contents in the *S. platensis* biomass grown in the different media are shown in Table 4 and Figure 3. The mean phycocyanin concentrations measured in biomass grown in CF-MLE-10, CF-MLE-15 and CF-MLE-20 were 0.75 ± 0.01; 0.77 ± 0.03 and 0.75 ± 0.01 mg L^{-1}, respectively (Figure 3). Although the phycocyanin content on the JM treatment was higher than that in the CF-MLE treatment, no significant difference was observed. The mean content of phycoerythrin and allophycocyanin, measured in the biomass grown in CF-MLE-15 was 0.28 ± 0.03 and 0.19 ± 0.001 mg L^{-1}, respectively (Figure 3). Like the phycocyanin content, the JM treatment had the highest concentration of phycoerythrin and allophycocyanin, which did not differ from the CF-MLE treatments. The productivity of phycocyanin, phycoerythrin and allophycocyanin of *S. platensis* in CF-MLE-15 was found to be similar to the standard medium. This result was significantly higher ($p < 0.05$) than the control treatment, which had a yield of 0.006 ± 0.001 g L^{-1} d^{-1}, 0.003 ± 0.001 g L^{-1} d^{-1}, and 0.002 ± 0.001 g L^{-1} d^{-1}, respectively (Table 5). The results for phycocyanin content differ from those of Simo *et al.* [13], who received 0.14, 0.20, and 0.05 mg L^{-1} for C-phycocyanin, allophycocyanin, and phycoerythrin, respectively, in a medium formulated with 8 g L^{-1} oil palm empty fruit bunch medium. These differences may be attributed to the essential role of potassium and nitrogen supplied by *Moringa oleifera* leaf extract and chicken feather in the medium. Indeed, activating enzymes involved in phycobiliprotein synthesis affect phycobiliprotein production in metabolic responses to changes in medium nutrient availability, as reported by Baslam *et al.* [32].

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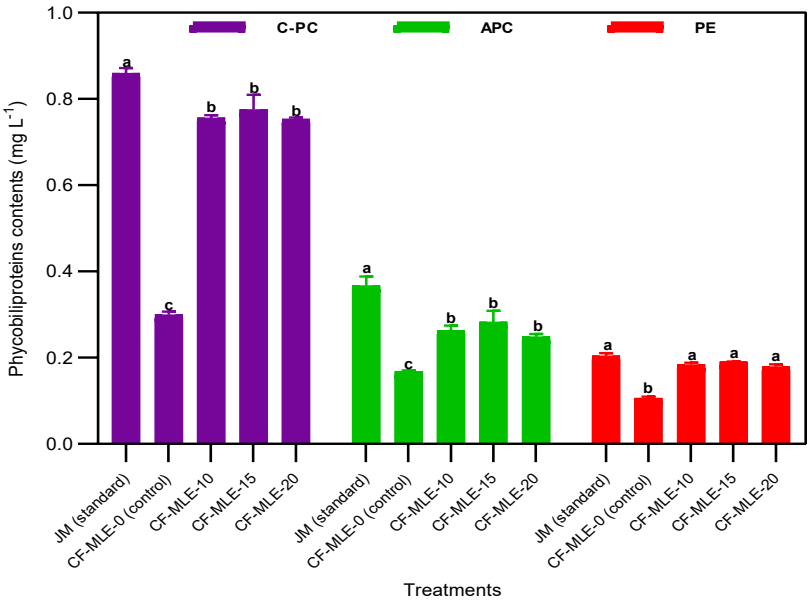

Figure 3. Phycobiliproteins content of *S. platensis* in chicken feathers medium supplemented with *M. oleifera* leaf extract (CF-MLE). The data are presented as means ± standard deviations. The bars represent the standard deviation. The means with matching superscript letters (a > b > c > d) within the same column are not significantly different (p > 0.05) according to the Tukey test.

Table 5. Chlorophyll (Chl a and b), total carotenoids (Cat), C-Phycocyanin (C-PC), Allophycocyanin (APC) and Phycoerythrin (PE) productivities of *S. platensis* after 27 days of cultivation in chicken feathers medium enriched with *M. oleifera* leaf extract (CF-MLE)

Culture medium	Treatments (g L^{-1})	P_{Chla} ($g.L^{-1}.d^{-1}$)	P_{Chlb} ($g.L^{-1}.d^{-1}$)	P_{Cat} ($g.L^{-1}.d^{-1}$)	$P_{C\text{-}PC}$ ($g.L^{-1}.d^{-1}$)	P_{APC} ($g.L^{-1}.d^{-1}$)	P_{PE} ($g.L^{-1}.d^{-1}$)
Jourdan (standard)	JM	0.15 ± 0.001^{a}	0.12 ± 0.002^{a}	0.08 ± 0.0003^{a}	0.08 ± 0.002^{a}	0.03 ± 0.003^{a}	0.02 ± 0.001^{a}
Chicken feathers supplemented with *M. oleifera* leaf extract	CF-MLE - 0 (control)	0.02 ± 0.002^{c}	0.01 ± 0.001^{b}	0.004 ± 0.0007^{d}	0.006 ± 0.001^{b}	0.003 ± 0.001^{b}	0.002 ± 0.001^{b}
	CF-MLE - 10	0.09 ± 0.001^{b}	0.09 ± 0.003^{a}	0.04 ± 0.0004^{c}	0.06 ± 0.001^{a}	0.02 ± 0.002^{a}	0.01 ± 0.001^{a}
	CF-MLE - 15	0.16 ± 0.001^{a}	0.11 ± 0.002^{a}	0.07 ± 0.0002^{a}	0.07 ± 0.005^{a}	0.03 ± 0.004^{a}	0.02 ± 0.002^{a}
	CF-MLE - 20	0.11 ± 0.002^{b}	0.10 ± 0.001^{a}	0.05 ± 0.0009^{b}	0.07 ± 0.001^{a}	0.02 ± 0.001^{a}	0.02 ± 0.002^{a}

The data are presented as means ± standard deviations. The bars represent the standard deviation. The means with matching superscript letters (a > b > c > d) within the same column are not significantly different (p > 0.05) according to the Tukey test.

Effects of chicken feathers medium supplemented with *Moringa oleifera* leaf extract on the protein and cysteine contents of *Spirulina platensis*

The protein content of *S. platensis* biomass grown in chicken feathers medium supplemented with different concentrations of *M. oleifera* leaf extract is presented in Figure 4 The protein content (94.5 ± 1.52 mg L^{-1}) was higher in CF-MLE-15 than in the other treatments and the control (39.3 ± 4.21 mg L^{-1}). We also recorded a high protein content (110.2 ± 6.14 mg L^{-1}) in Jourdan's standard medium. Compared with the control, the addition of *M. oleifera* leaf extract to the chicken feathers medium significantly increased the protein content of *S. platensis* biomass in all the treatment groups ($p < 0.05$). Therefore, mineral elements such as nitrogen, magnesium and potassium in CF-MLE may be essential cofactors for protein production [35]. The cysteine content of *S. platensis* grown on CF-MLE-15 (12.5 ± 1.14 mg L^{-1}) and Jourdan standard medium (14.1 ± 0.81 mg L^{-1}) was significantly higher ($p < 0.05$) than that of the other treatments and the control (3.8 ± 0.27 mg L^{-1}). These cysteine contents results were lower than those reported by Magwell *et al.* [36] who reported 0.16 g L^{-1} cysteine in $MgSO_4$-enriched medium. This difference could be explained by the composition of the culture medium in which the microalgae grew.

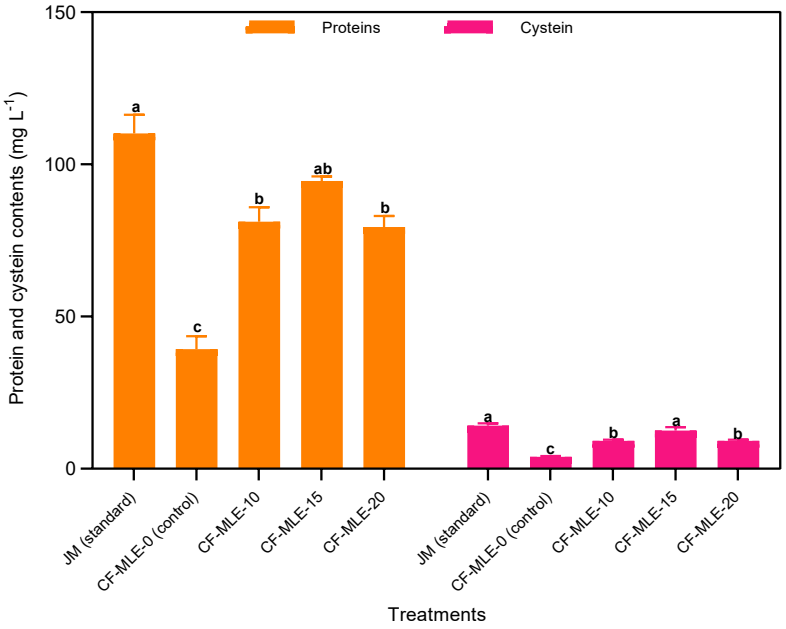

Figure 4. Protein and cysteine contents of *S. platensis* in Chicken feathers medium supplemented with *M. oleifera* leaf extract (CF-MLE). The data are presented as means ± standard deviations. The bars represent the standard deviation. The means with matching superscript letters (a > b > c > d) within the same column are not significantly different ($p > 0.05$) according to the Tukey test.

Conclusion

This study examined the potential of chicken feathers and *Moringa oleifera* leaf extract as an eco-friendly medium for the production biomass and photosynthetic pigments from *S. platensis*. The results revealed that the biomass production, protein and photosynthetic pigment contents were related to the concentration of *M. oleifera* leaf extract in chicken feather media. The highest biomass, protein and photosynthetic pigment content were observed in the chicken feather medium supplemented with 15 g L^{-1} *M. oleifera* leaf extract, in addition to the standard medium. Thus, chicken feathers and *M. oleifera* leaf extract have emerged as promising and eco-friendly approaches for co-producing biomass and pigments from *S. platensis* for pharmaceutical, nutraceutical, food and cosmetic applications. Future research should investigate the life cycle assessment and the CF-MLE-15 medium scalability in photobioreactors for industrial *Spirulina platensis* production performance, cost, and feasibility.

List of Abbreviations

APC	Allophycocyanin
PAPC	Allophycocyanin Productivity
Cat	Carotenoid
PCat	Carotenoid Productivity
CF	Chicken Feathers
CF-MLE	Chicken Feathers medium supplemented with *Moringa oleifera* Leaf Extract
Chl a	Chlorophyll a
PChla	Chlorophyll a Productivity
Chl b	Chlorophyll b
PChlb	Chlorophyll b Productivity
C-PC	C-phycocyanin
PCPC	C-phycocyanin Productivity
pH	Hydrogen potential
JM	Jourdan Medium
LED	Light Emitting Diode
MLE	*Moringa oleifera* Leaf Extract
OD	Optical Density
PE	Phycoerythrin
PPE	Phycoerythrin Productivity

Author Contributions

Conceptualization and design: W.F.O., M.P.F.R., L.C. and S.C. Methodology: W.F.O., S.C., M.P.F.R., L.C., T.D.K., N.B.G. and N.N.P.L. Figures creation and design: W.F.O., M.P.F.R. and S.C. Writing - original draft

preparation: W.F.O., S.C., M.P.F.R., L.C., N.B.G and T.D.K. Edited, reviewed manuscript and provided with critical input and corrections: W.F.O., S.C. and M.P.F.R. Supervision: W.F.O., S.C., M.E. and L.L.G. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

Data supporting the results of the current study are available upon request from the corresponding author.

Consent for Publication

Not Applicable.

Conflicts of Interest

The authors state that there no conflicts of interest regarding this manuscript.

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